HIGH TEMPERATURE TOLERANCE AND THYROID ACTIVITY IN THE TELEOST FISH, TANICHTHYS ALBONUBES [1]

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Upper lethal temperatures have been ascertained for many species of fish (Loeb and Wasteneys, 1912; Hathaway, 1927; Sumner and Doudoroff, 1938; Brett, 1941, 1944, 1946, 1952; Doudoroff, 1942; Fry *et al.*, 1942, 1946). Different species, when tested under comparable conditions, exhibit characteristic diverse high-temperature death points which are often clearly related to the conditions of life to which the species are adapted. However, the precise mechanism by which death is caused at high temperature in ectotherms is still not understood (Fisher, 1958; Precht, 1958). The experiments of Fortune (1955) led to the conclusion that increased activity of the thyroid gland with increasing environmental temperature may be an important factor in the thermal death of fishes. Fortune reported that specimens of *Phoxinus* (*phoxinus*) *laevis* kept in .05% thiourea solution at 23° C. for three days and then subjected to a 10° temperature rise over a two-day period were able to survive this treatment and to live indefinitely at 33° C. On the other hand, specimens given no thiourea treatment during the three-day period of acclimation at 23° C. all succumbed by the time the temperature reached 24° C. Inhibition of thyroid activity by thiourea treatment thus enabled *Phoxinus* to tolerate a temperature 9° C. above that which would otherwise have been lethal. Fortune also reported that the thermal range of *Lebistes reticulatus* could be similarly extended by thiourea treatment.

Earlier work by Evropeitzeva (1949) on thiourea-treated fry of *Coregonus* is in agreement with Fortune's findings but several other investigations have given diametrically opposed results. LaRoche and Leblond (1954) found that the parr of *Salmo salar* thyroidectomized with radioiodine had a lower tolerance for high temperature than did untreated controls. Auerbach (1957), working with *Lebistes reticulatus, Platys variatus,* and *Leuciscus rutilus,* and Theobald (1959) with *Gambusia affinis* found that thiourea treatment decreased the high-temperature tolerance in these species. Suhrmann (1955) reported that the goldfish, *Carassius vulgaris,* has an increased high-temperature tolerance if kept in thiourea at a low acclimation temperature but has a decreased tolerance if the acclimation temperature is high.

This paper reports the results of a study of the effects of thiourea treatment upon high-temperature tolerance and upon thyroid histology in the cyprinid, *Tanichthys albonubes.* Effects upon pituitary histology, which were also investigated, will be considered in a separate publication.

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Materials and Methods

Tanichthys albonubes Lin, the common whitecloud, is a small tropical oviparous fresh-water fish. A total of about 300 specimens was used in this study. The average length of the fish used, measured from the tip of the snout to the hypural plate, was 2.6 cm. ± 0.2 cm.

During the phases of the experiments described below, the control whiteclouds were kept in dechlorinated tap water and the experimental fish were kept in 0.05% thiourea contained in either 5-gallon aquaria or polyethylene pails. These media received continual aeration and were changed twice a week. The fish were fed daily with dry tropical fish food. Prior to the actual experiments several series of pilot experiments were carried out before adopting the subsequent methods regarding the concentration and period of treatment with thiourea, the acclimation temperature to be used, and the method of determining the upper lethal temperature.

1. *Pre-acclimation period*

The pre-acclimation period was designed to bring about hypofunction of the thyroids of the experimental fish. To accomplish this, no more than 75 experimental fish were placed in a 5-gallon aquarium containing 18 liters of 0.05% thiourea and kept at room temperature (21° C.). An equal number of controls were also kept at room temperature in an equal volume of water in a similar aquarium. This phase was continued for 30–40 days. At the end of this period, histological examination of the thyroids of a few treated animals revealed that thyroid hypofunction had been effected.

2. *Acclimation at low temperature*

After the pre-acclimation treatment, the control and experimental whiteclouds were kept in 11-quart polyethylene buckets containing thiourea solution or water. Each bucket contained no more than 40 fish. The buckets were placed in a 50-gallon Aminco Laboratory Bath (refrigerator type) regulated to maintain a temperature of 15.0° C. ± 0.05° C., and kept there from 40 to 60 days. During the pre-acclimation and acclimation phases, fish mortality was negligible.

3. *Upper lethal temperature determination*

A common method for determining the upper lethal temperature of fish is to directly transfer the test animals from the acclimation tank to the high-temperature tank and then to calculate the temperature at which half of the animals die in a given period of time. The periods of exposure to the high temperature vary widely in different studies. A number of investigators have used a 24-hour period (Black, 1953; Theobald, 1959). Following these authors, the lethal temperature in this study was considered to be that temperature at which 50% of the fish succumbed and 50% survived after 24 hours' exposure. High temperatures at which the whiteclouds were tested ranged from 29.5° C. to 31.5° C. The water baths used to test the fish at high temperatures were Aminco Laboratory Baths which could maintain water temperatures within 0.05° C.

It was observed in preliminary experiments that when whiteclouds were directly transferred from the acclimation tank to a high-temperature tank, symptoms of shock, such as rapid movement of the opercula, quick spiral swimming, and partial or total loss of equilibrium, occurred. To minimize this shock the method was modified as follows. Water baths were set at the high temperatures at which fish were to be tested. Samples of control and experimental fish (4–6 fish per sample) from the acclimation bath were placed in small polyethylene pails containing either water or 0.05% thiourea already cooled to 15.0° C. The pails were then transferred to a hot-water bath set at 45.0° C., and left there until the temperatures at which the fish were to be tested (29.5° C. to 31.5° C.) were reached. This required 15 ± 0.5 minutes. The pails were then placed in the appropriate water baths set at the temperature at which that particular group of fish was to be tested. The fish were left at this temperature for 24 hours. This substitution of a method of increasing the temperature over a period of 15 minutes for the direct transfer method originally used eliminated all signs of shock.

4. *Methods of fixation and staining*

After 24 hours' exposure to the upper lethal temperature, surviving control and experimental fish and an identical number of specimens from corresponding solutions in the 15.0° C. acclimation bath were removed and anesthetized in 1/1000 tricaine-methane-sulfonate (MS-222). The MS-222 was warmed or cooled to the temperature from which the fish were taken. The lower jaws of these fish were fixed in Bouin's fluid and embedded in paraffin. Records were kept concerning the sex and maturity of all specimens. Serial sections of the thyroid glands from control and experimental fish from the same sex group and from all three phases of the experiment were stained simultaneously. The thyroids were stained in Gomori's chrome-alum-hematoxylin-phloxine.

Observations

1. *Thyroid histology*

The thyroid histology of the experimental fish was significantly different from that of the control animals. The thyroids of control fish at the end of the various phases of the experiment showed no significant differences. Similarly, no significant differences were observed in the thyroid histology of the thiourea-treated fish after each of three experimental phases. The descriptions which follow are based on histological observations of the thyroids of control and experimental fish killed at the end of the acclimation phase of the experiment.

Examination of serial sections of individuals from the control groups showed that the thyroid of the whitecloud belongs to the non-encapsulated diffuse type found in most teleosts (Fig. 1). The follicles are scattered in the connective tissue beneath the floor of the pharynx but tend to accumulate around the ventral aorta and the aortic arches. These follicles are roughly spherical in shape. The average number of follicles found in a representative cross-section through the thyroid region of control fish is 9. Occasionally small capillaries are seen in contact with some of the follicles. The follicular epithelium is mostly squamous, although some of the small follicles have cuboidal to low columnar epithelium, the average height being 3.0 microns (Fig. 2). The squamous cells contain little cytoplasm, and their flattened, dark-staining nuclei occupy most of the cell. The cuboidal epithelial cells have round, usually basally located, nuclei and a small amount of slightly basophilic cytoplasm. No colloid droplets or vacuoles

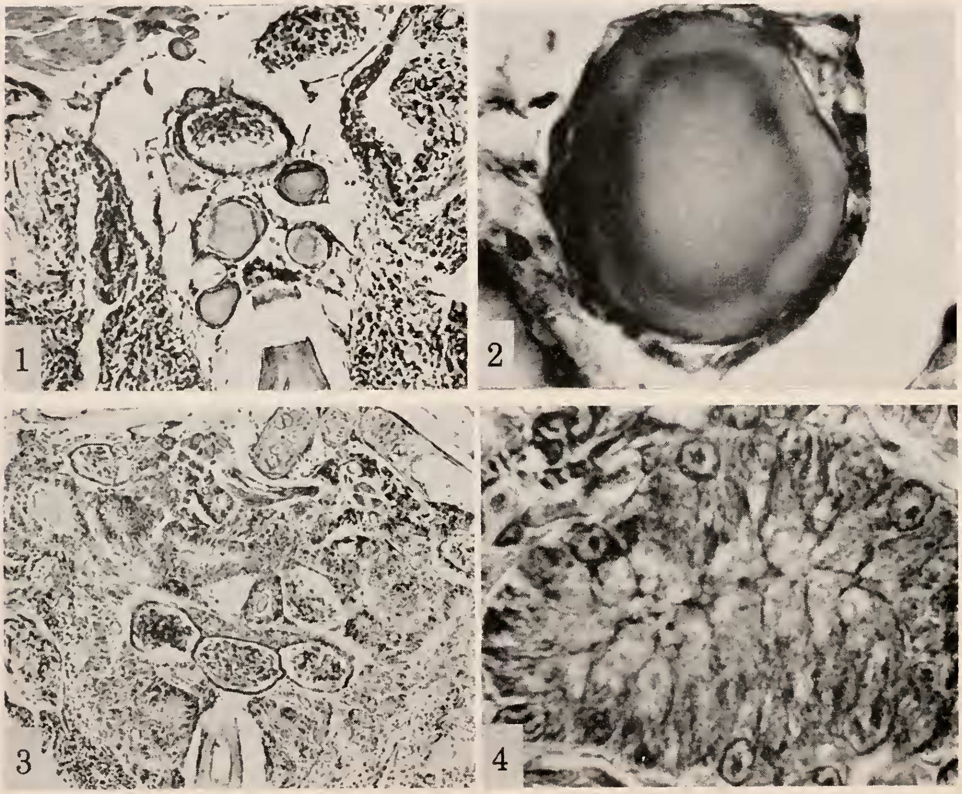

FIGURE 1. Section through thyroid region of an untreated control kept at room temperature (21° C.) for 30 days, then at 15° C. for an acclimation period of 60 days. 50 ×.

FIGURE 2. A single thyroid follicle from the specimen shown in Figure 1. 485 ×.

FIGURE 3. Section through thyroid region of a fish kept in 0.05% thiourea throughout a preacclimation period of 30 days at 21° C. and an acclimation period of 60 days at 15° C. 50 ×.

FIGURE 4. A single thyroid follicle from the specimen shown in Figure 3. 485 ×.

were observed in any of the follicular cells of the control thyroids. Except for a few of the small, more active follicles, the follicular lumina are large and filled with acidophilic, homogeneous, or granulated, non-vacuolated colloid.

Examination of the thyroids of thiourea-treated fish reveals a histological picture quite different from that of the controls (Fig. 3). A great increase in the number and size of follicles has occurred so that the follicles are more compactly arranged and occupy a large portion of the sub-pharyngeal region. The average number of follicles found in a representative cross-section through the thyroid region of experimental fish is 25. The follicular epithelium is columnar and averages 19.5 microns in height. The cytoplasm of the thyroid epithelium contains large vacuoles and intracellular colloid droplets not found in controls (Fig. 4). Large, round, vesicular, basally located nuclei containing prominent nucleoli are found in most of the follicular cells. Many follicles are collapsed while those which are not contain only a small amount of basophilic, vacuolated colloid. A hyperemic state is evidenced by the increase in the number and size of interfollicular capillaries and other small blood vessels when compared with the controls.

Table I

Survival of fish previously acclimated at 15° C. and then exposed for 24 hours to the high temperatures indicated

Test		29.5°	30.0°	30.5°	31.0°	31.5°
Controls	#1			5/6	6/6	0/3
	#2				3/6	0/6
	#3			3/6	3/5	
	#4				3/6	
	#5				2/6	
	#6				3/6	
	#7				0/6	
	#8				6/6	
Totals				8/12 (67%)	26/47 (55%)	0/9
Thiourea-treated	#1	3/6	3/4	0/4	0/4	
	#2		4/6	2/6		
	#3		3/6	2/6		
	#4		3/6	1/5		
	#5		4/5	0/5		
	#6		3/6			
	#7		1/6			
	#8		3/6			
Totals		3/6 (50%)	24/45 (53%)	5/26 (19%)	0/4	

2. *Upper lethal temperature*

Based on the method described above, the upper lethal temperature for control *Tanichthys albonubes* was found to be 31.0° C., while that for the experimental fish was 30.0° C. The results of the experiment are summarized in the accompanying table. The upper half of the table contains the data obtained for control fish while the lower half contains the data for experimental fish. The figures in the uppermost part of the table indicate the various high temperatures to which the fish were exposed. The equations under each temperature show the number of fish per sample which survived at that particular temperature for 24 hours. For example, in test #1 of the controls, 5 out of 6 fish survived at 30.5° C., 6 out of 6 survived at 31.0° C., and none out of three survived at 31.5° C. when left at these temperatures for 24 hours. In test #2 of the controls, three out of 6 fish survived 24 hours' exposure at 31.0° C., while all of the 6 fish placed in 31.5° C. died within 24 hours. Since the early tests indicated that the upper lethal temperature for the whitecloud was 31.0° C., the majority of the tests were made at this temperature. Totals of these tests show that 8 out of 12 fish, or 67%, survived 24 hours at 30.5° C., 26 out of 47, or 55%, survived 31.0° C., while 24-hour exposure to 31.5° C. proved lethal for all fish kept at this temperature. Similar comparisons may be made for the experimental animals by examining the lower part of the table. A comparison of the results obtained for controls and experimentals indicates that the controls had a greater capability to withstand high temperature than did the fish treated with thiourea. It should be mentioned that there was no significant difference in the survival in males and females.

Discussion

Piscine thermal tolerance extremes depend on previous acclimation temperatures. This fact is evident from the numerous thermal studies done on fish since the time of Loeb and Wasteneys (1912). The rate at which acclimation occurs, however, seems to differ widely in different fish. Some authors (Doudoroff, 1942; Brett, 1944, 1946) have found that gain of heat tolerance is more rapid than loss in the process of acclimation. Tsukuda (1960), on the other hand, reported that changes in cold tolerance are almost parallel to changes in heat tolerance in the guppy, *Lebistes reticulatus,* with both processes being relatively slow. Using approximately the same temperature change intervals Doudoroff (1942) and Brett (1944) found for *Girella nigricans* and *Phimephales pomelas,* respectively, that a period of 20 days or more was required to completely acclimate these fish at low temperatures. About 35 days are required to acclimate male guppies at low temperatures (Tsukuda, 1960). Although no rate of acclimation was ascertained for the whitecloud in the present experiments, in view of the findings of Doudoroff, Brett and Tsukuda, and of the relative stability obtained in the lethal temperature for the whitecloud, the 40–60 days' exposure to 15.0° C. seems to have been a sufficient period to bring about a stable physiological condition at the low temperature.

Under the conditions of this experiment the high-temperature death point for controls of the cyprinid, *Tanichthys albonubes,* was found to be 31.0° C. Brett (1956) tabulated the reported lethal temperatures for a number of species of fish. Members of the Cyprinidae recorded by Brett have upper lethal temperatures ranging from 28.9° C. to 32.8° C. Hence, the upper lethal temperature ascertained for the whitecloud here is in agreement with the range of upper thermal limits recorded for other members of the family.

With a few exceptions, the thyroid of teleosts consists of unencapsulated follicles scattered individually or in small groups in the connective tissue along the ventral aorta and branchial arteries in the lower jaw of the fish. The thyroid morphology of *Tanichthys albonubes* is consistent with this general pattern. This diffuse nature of the teleost thyroid renders complete surgical extirpation of the gland impossible. To study the effect of hypothyroidism in these animals one must have recourse to chemical inhibitors or to thyroidectomy by radioiodine. Thiourea has been used on teleosts by a number of investigators (Scott, 1953; Fortune, 1953, 1955, 1956, 1958; Frieders, 1949, 1954; Suhrmann, 1955; Auerbach, 1957; Theobald, 1959; and others). Common effects of thiourea treatment on the thyroid histology of fish include increased vascularization in the thyroid area, hyperplasia and hypertrophy of the follicular epithelium, and a loss of stored colloid. The degree to which these results are achieved depends on the species of fish used, the concentration of thiourea, the length of the treatment and other factors. This hyperactive appearance of the thyroid after thiourea treatment may be explained on the basis of the thyroid-pituitary relationship which exists in fish as in other vertebrates. Thiourea prevents the thyroid from synthesizing its hormone so that, as soon as the supply of circulating hormone present at the beginning of thiourea treatment falls below a certain level, the pituitary begins to secrete thyrotrophin to activate the thyroid to produce more hormone. The thyroid, however, because of continued treatment, remains hypofunctional despite the fact that it is hyperactive. Nevertheless, in some species under certain conditions, prolonged treatment with antithyroid drugs may result in an "escape" from thyroid inhibition (Pickford and Atz, 1957). Frieders (1949) observed that the hyperplastic thyroid of *Trichogaster trichopterus* resulting from 0.0025% thiourea treatment at room temperature returned to a normal state after the fifth week of treatment. Similar reactions to thiourea were shown by Fortune (1958) to exist in *Phoxinus laevis*. The hyperemic, hyperplastic and hypertrophic condition of the thyroid following thiourea administration in the present work is clear evidence that the thyroid of the whitecloud was quite responsive to thiourea treatment. Since this hyperactive state persisted from the beginning of the acclimation at low temperature to the end of the experiment, it is concluded that there was no "escape" from the thyroid inhibition, and that the thyroid hormone was totally or to a great degree suppressed in the experimental animals during this time, a desired condition for the experiment.

The dramatic results obtained by Fortune (1953, 1955) served as a basis for the hypothesis that decreased thyroid function in teleosts at high temperature is a significant factor in causing death at the lethal temperature. Fortune (1955) kept *Phoxinus (phoxinus) laevis* in thiourea at 23° C. for three days and then subjected them to a rise in temperature to 33.0° C. over a period of two days. The experimental fish survived indefinitely at this increased temperature and appeared normal, while non-thiourea-treated fish died under such conditions between 23.0° C. and 24.0° C. Although no data were given, Fortune reported in the same paper that the thermal range of *Lebistes reticulatus* could also be extended by thiourea treatment.

Other experiments involving high-temperature tolerances and thyroid hypofunction in teleosts have produced conflicting results. Evropeitzeva (1949) reared six-day-old fry of *Coregonus lavaratus ludoga* for 17 days in thiourea at room temperature and then exposed 100 control and experimental animals to 29.0° C. for 5 minutes. Ninety-seven per cent of the control animals died while 97% of the experimental animals survived the 5-minute exposure. These results seem to agree with Fortune's hypothesis. The studies of LaRoche and Leblond (1954) and Auerbach (1957) on other young fish, however, oppose the work of Evropeitzeva. LaRoche and Leblond thyroidectomized young Atlantic salmon, *Salmo salar*, by radioiodine and found that thyroidectomy impaired the ability of these salmonid parr to withstand rising temperatures (5.0° C. to 10.0° C.), while the thyroidectomized fish receiving thyroid material in their diet survived such rising temperatures. Auerbach acclimated young *Xiphophorus helleri* at 15.0° C. and 25.0° C., and then put some of the fish in 0.15% thiourea at the same temperatures for 14 days. She tested the control and experimental fish at high temperatures and found that the cold- and warm-adapted controls evidenced heat coma at higher temperatures than did the cold- and warm-adapted thiourea-treated fish.

Using adult fresh-water fish, Suhrmann (1955) decreased thyroid function in *Carassius vulgaris* by keeping these fish in thiourea at 5.0° C. and 26.0° C., and then found that heat coma occurred in the cold- and warm-acclimated fish at 31.4° C. and 35.2° C., respectively. By comparing her results with the findings of Christophersen and Precht (1952) for untreated *Carassius vulgaris* kept at the same acclimation temperatures, she deduced that thiourea increased upper temperature tolerance for cold-acclimated fish, but decreased it for the warm-acclimated ones.

The work of Auerbach (1957) and Theobald (1959) on other adult fresh-water fish does not agree with Fortune's results. Auerbach reported that controls for *Lebistes reticulatus* and *Platy variatus* acclimated at 25.0° C. and 15.0° C. had higher heat coma temperatures than experimental animals acclimated at the same temperatures but subjected to a 14-day treatment of 0.15% thiourea. Theobald administered various concentrations of thiourea or thyroid-stimulating hormone (TSH) to *Gambusia affinis* for 5 weeks at 25.0° C., and then acclimated the fish to 30.0° for one week. He found that the upper lethal temperatures for control, thiourea-treated, and TSH-treated animals were 37.4° C., 35.6° C. and 37.9° C., respectively.

Auerbach (1957) reported that the marine fish *Leuciscus rutilus,* if treated with thiourea, could not stand as high temperatures as non-treated fish. She kept a group of this species at 5.0° C. for 2–4 weeks and a group at 20.0° C. for 1–2 weeks and then put half of each group in 0.15% thiourea for 14 days at these temperatures. She found that cold-adapted controls had a heat coma temperature range of 28.1° C. to 29.0° C., while the cold-adapted thiourea-treated fish had a heat coma temperature range of 26.3° C. to 27.0° C. The warm-adapted controls and thiourea-treated fish had heat coma ranges of 29.7° C. to 30.8° C. and 27.6° C. to 28.4° C., respectively.

The upper lethal temperature for whiteclouds exposed to 0.05% thiourea under the conditions of the present experiment was found to be 30.0° C., whereas the upper lethal temperature for untreated control fish was 31.0° C. Thus, these results are contrary to those of Fortune (1955) for *Phoxinus* and *Lebistes,* and indicate that thiourea treatment slightly decreased the ability of *Tanichthys albonubes* to withstand high lethal temperatures.

Summary

1. The thyroid of *Tanichthys albonubes* was rendered hypofunctional by 30–40 days' immersion in 0.05% thiourea. This treatment affected the thyroid histology to a significant degree, resulting in hyperemia, follicular hyperplasia, and cellular hypertrophy with a loss of stored colloid.

2. After each group had been acclimated at 15.0° C., the upper lethal temperatures were determined for the thiourea-treated and control fish. The upper lethal temperature of the controls was 31.0° C. and that of the experimentals was 30.0° C. This indicates that in this species decreased thyroid function does not result in increased high-temperature tolerance but, in fact, slightly decreases it.

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